Tropical Biomedicine 41(4): 441-449 (2024)
https://doi.org/10.47665/tb.41.4.004

RESEARCH ARTICLE

Larvicidal activity of methanolic and aqueous extracts of *Lavandula dentata* and *Nerium oleander* flowers against the vector of avian malaria *Culiseta longiareolata*

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ARTICLE HISTORY

Received: 23 March 2024
Revised: 9 July 2024
Accepted: 10 July 2024
Published: 31 December 2024

ABSTRACT

In light of the adverse effects of chemical insecticides on the environment and human health, as well as the development of mosquito resistance to them, this study explores the potential of methanol and aqueous flower extracts from *Lavandula dentata* and *Nerium oleander* as bioinsecticides against *Culiseta longiareolata* mosquitoes. Additionally, it aims to assess the impact of these extracts on enzymatic biomarkers and biochemical composition of fourth instar larvae of *Culiseta longiareolata.*Qualitative analysis revealed the presence of flavonoids, terpenes, gallic and catechic tannins in both plant extracts. Sterols and quinones were exclusively found in *L. dentata*, while saponins were identified in *N. oleander*. methanolic extracts demonstrated higher yields compared to aqueous extracts. The results showed significant larvicidal activity for all the tested extracts, the LC_{50} values after 24 hours are: 463.8; 338.1; 760.9 and 99.64 ppm for the aqueous and methanolic extract of *L. dentata* and *N. oleander* respectively. This proves that the methanolic extracts of *N. oleander* and *L. dentata* have higher efficacy against *C. longiareolata* compared to the aqueous extracts. Enzymatic activity assays conducted on larvae treated with various extracts, particularly at LC_{25} and LC_{50} concentrations, indicated an induction of the detoxification system, evidenced by increased glutathione S-transferases (GSTs) and catalase activity. Morphometric analysis revealed a significant reduction in the growth of fourth-stage larvae treated with these extracts. As well as a notable decrease of a percentage ranging from 44.23 to 83.30% in protein content; a reduction between 38.15-78.57% in carbohydrates and a percentage decrease of 23.85 to 63.58% in lipids. These findings suggest that the observed effects may be attributed to the stressful conditions imposed by the treatment, necessitating additional energy supply from carbohydrate and lipid metabolism in the larvae.

Keywords: Plant extracts; larvicidal activity; GSTs; catalase; biochemical composition.

INTRODUCTION

Various diseases, regardless of their tropical nature, are either caused by arthropods or transmitted by them (Khaligh *et al*., 2020). The order Diptera is the most important in terms of disease vectors, mainly because of the family Culicidae (mosquitoes) that transmit various diseases such as malaria, filariasis, yellow fever, West Nile fever, Japanese encephalitis, Rift Valley fever, etc (Bisanzio *et al*., 2011; Schoener *et al*., 2017; Vivekanandhan *et al*., 2021; Hegazy *et al*., 2022; Bauer, 2023; Onen *et al*., 2023).

Culiseta longiareolata (Macquart, 1838) is the most widely distributed species in Algeria along with *Culex pipiens* (Linnaeus, 1758). On the veterinary and ecological sides, avian malaria and different arboviruses are hugely interesting diseases; of which *C. longiareolata* is the main vector (Seidel *et al*., 2013). *C. longiareolata*, which is known as a common bird-bitten species. In laboratory studies, avian malaria parasites completed their development in *C. longiareolata* females. Endemic avian malaria produces deleterious effects on birds, affecting their health status, survival probability and reproductive success (Garrigós *et al*.,2024). Given the confined habitat of mosquitoes, controlling them during their larval stage becomes imperative (Dris *et al*., 2017a; Bouabida & Dris, 2020; Dris *et al*., 2021; Bouabida & Dris, 2022b). However, the efficacy of chemical insecticides in controlling mosquito populations has dwindled due to the development of resistance, besides posing adverse effects on aquatic ecosystems owing to their non-biodegradable nature and propensity for biological accumulation (Dahmana & Mediannikov, 2020; Grabsi *et al*., 2023; Njoroge *et al*., 2023). Consequently, there is a pressing need for novel mosquito control methods that are eco-friendly, cost-effective and non-toxic to non-target organisms (Oliveros-Díaz *et al*., 2022; Seghier *et al*., 2023). Plant extracts and their secondary metabolites have emerged as promising alternatives for mosquito larvae control owing to their insecticidal properties, biodegradability, and cost-effectiveness

(Pratheeba *et al*., 2019). Botanical compounds harbor numerous active ingredients capable of disrupting the mosquito life cycle and mitigating the nuisance they pose to humans (Abagli & Alavo, 2020).

Nerium oleander L. (Apocynaceae), a flowering shrub native to Mediterranean tropical and subtropical regions, exhibits various pharmacological properties including antibacterial, antimicrobial, antifungal, anti-inflammatory, antioxidant, and insecticidal activities (MostaqulHuq *et al*., 1999; Erdemoglu *et al*., 2003; Hussain & Gorski, 2004; Derwic *et al*., 2010; Mohadjerani, 2012; Bagari*et al*., 2013; Siddiqui *et al*., 2016; Al-Jameeli, 2021; Elimem *et al*., 2022). The use of *N. oleander* leaves has dermal, digestive and cardiovascular toxicities (Belhaj *et al*., 2021). Several studies have documented its mosquito larvicidal activity against various genera including *Culex, Anopheles* and *Aedes* (Lokesh *et al*., 2010; Raveen *et al*., 2014; El-Akhal *et al*., 2015; Al-Hakimi *et al*., 2022).

Lavandula dentata L. (Lamiaceae) is another plant rich in phytochemicals belonging to different chemical families such as phenolic acids, flavonoids, and terpenoids (Bouyahya *et al*., 2023). Pharmacological investigations have revealed its antibacterial, antifungal, antioxidant, anticancer, anti-inflammatory, and insecticidal properties (Algieri *et al*., 2016; Dris *et al*., 2017b; Al Sufyani *et al*., 2019; Dammak *et al*., 2019; Pereira *et al*., 2019; Imen *et al*., 2021; El-Akhal *et al*., 2021; Wagner *et al*., 2021; Alqudah *et al*., 2023; Dobros *et al*., 2023; El Abdali *et al*., 2023; Jilali *et al*., 2023; Terfi *et al*., 2023). Belhaj *et al*. (2021) indicate that all *L. dentata* parts have no toxic activities. Therefore, this study aims to evaluate the larvicidal activity of methanolic and aqueous flower extracts of *N. oleander* and *L. dentata* against *Culiseta longiareolata* mosquitoes and to explore their effects on enzymatic biomarkers and biochemical composition.

MATERIALS AND METHODS

Plant material, extraction and phytochemical screening

The *Lavandula dentata* and *Nerium oleander* flowers (Figure 1) were gathered from the Tebessa region of Algeria in November and December 2021 (GPS: 25°222 202 2 N, 8°142 82 2 E and 891m altitude). The plants were authenticated by a plant taxonomist from the Department of Living Beings, Faculty of Exact Sciences and Natural and Life Sciences, Echahid Cheikh Larbi Tebessi University, Tebessa, Algeria. The flowers were meticulously washed and air-dried for 15 days until a constant weight was achieved. Subsequently, they were ground to a particle size ranging from 200 to 500µm and subjected to extraction with water and 70% methanol under agitation at room temperature for 24 hours. The obtained extracts were evaporated under reduced pressure, resulting in dried residues that were used for various assays (Gheraibia *et al.,* 2000). The percentage yield of the extracts was calculated using the formula: R (%) = (Mass of extract / Mass of plant powder) × 100.

The qualitative chemical composition of the crushed flowers was determined using standard methods (Harborne, 1998), including the identification of flavonoids, tannins, terpenes, alkaloids, sterols, saponins, and quinones.

Larvicidal test

The larvicidal activity of the methanolic and aqueous extracts of *Lavandula dentata* and *Nerium oleander* was evaluated against fourth instar larvae of *Culiseta longiareolata* following the guidelines of the World Health Organization (WHO, 2005). Larvae were fed a mixture of biscuits and yeast powder (3v/1v) for two days prior to the experiment. Twenty-five larvae were exposed to various concentrations of the extracts (ranging from 60 to 660ppm), while dechlorinated water was used as a negative control. Mortality rates were recorded after 24, 48, and 72 hours, with each experiment conducted in five repetitions.

Glutathione S- transferase assay

The activity of glutathione S-transferases (GSTs) was determined using the method described by Habig *et al*. (1974). This involved the conjugation reaction between GST and CDNB substrate in the presence of glutathione, with measurements taken at 340nm using a spectrophotometer. The effects of different concentrations (LC_{25} and LC_{50}) of the extracts on GST activity were evaluated at intervals of 24, 48, and 72 hours post-exposure. The decapitated larvae were homogenized in 0.1M sodium phosphate buffer at pH 6, centrifuged for 30min at 14000rpm/min and the GST activity was assessed as described by Dris *et al*. (2017a).

Catalase enzyme activity

Catalase activity was assessed according to the method outlined by Claiborne (1985). The reduction of hydrogen peroxide (H_2O_2) to water and oxygen in the presence of catalase was measured spectrophotometrically at 240nm. The control and treated fourth instar larvae of *C. longiareolata* are sampled at different times (24, 48, 72 hours), the test is conducted with 4 replicates each comprising 10 individuals. The larvae are homogenized in 1mL of phosphate buffer (100mM, pH 7.4), then centrifuged at 15,000 rpm for 10min. The recovered supernatant will serve as a source of enzyme. An aliquot of 50µL of the supernatant to which 750µL of phosphate buffer (100mM, pH 7.4) are added. After stirring, the reading is taken with a spectrophotometer. Absorbances are read after every 5 seconds for 30 seconds at a wavelength of 240 nm against a blank with 800µL of phosphate buffer (100mM, pH 7.4), and 200µL of H_2O_2.

Effect of extracts on growth of *Culiseta longiareolata*

The fourth instar of newly exuviated *C. longiareolata* larvae were treated with aqueous and methanolic extracts of *L. dentata* and *N. oleander* at its LC_{25} and LC_{50}. The weight growth of individuals

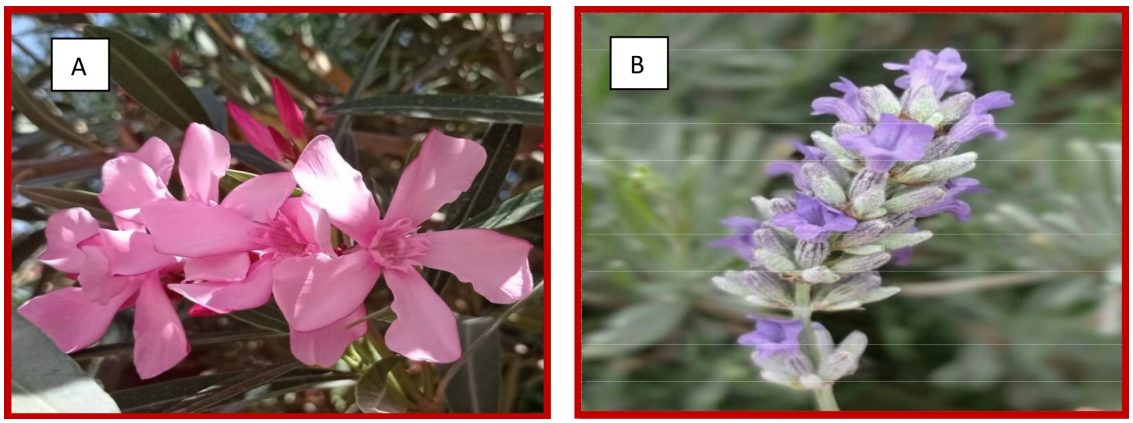

Figure 1. Flower's photos of *N. oleander* (A) and *L. dentata* (B).

of treated and untreated larvae was determined using a precision balance on 4 repetitions of 10 individuals.

Extraction and assay of biochemical constituents

Biochemical constituents (proteins, carbohydrates, and lipids) were extracted following the procedure described by Shibko *et al*. (1966) and quantified as per the method by Dris *et al*. (2017a). In brief, the control and LC_{25} and LC_{50} treated larvae were sampled at 4 replicates and stored in 1 mL of 20% TCA (trichloroacetic acid). After ultrasonic homogenization and according to the method of Duchateau & Florkin (1959) and after centrifugation (5000rpm at 4°C for 10min), the supernatant I obtained will be used for the determination of total carbohydrates. To pellet I, 1mL of ether/chloroform mixture (1V/1V) was added and after a second centrifugation (5000 rpm, 10min), a supernatant II was obtained which will be used for the lipid assay (Goldsworthy *et al*., 1972). A pellet II, dissolved in sodium hydroxide (0.1N), will be used for the protein assay according to Bradford (1976).

Statistical analysis

The results were given as the mean ± SE. After verifying the conditions of normality and homogeneity of variance, a one- and two-way analysis of variance are used followed by a multiple comparison test of means (Tukey and Dunnett test). Statistical analysis was performed using SPSS software (version 23) and GRAPH PAD PRISM 7.

RESULTS

Phytochemical screening and determination of extraction yield

The results of the phytochemical screening conducted on *L. dentata* and *N. oleander* are presented in Table 1. The qualitative assessment revealed the presence of flavonoids, gallictannins, catechic tannins, and terpenes, alongside the absence of alkaloids in both species. Additionally, sterols and quinones were exclusively identified in *L. dentata*, while saponins were solely detected in *N. oleander*. Table 2 presents the extraction yields obtained from various solvents. The results demonstrate that the choice of solvent significantly influenced the yield of extracts, with methanolic extracts yielding higher quantities compared to aqueous extracts, relative to the initial weight of the plant material used.

Toxicity test

The toxicity assessment was conducted on the fourth larval stage of *Culiseta longiareolata* using varying concentrations of methanolic and aqueous extracts derived from *L. dentata* and *N. oleander*: 60, 130, 200, 330, 460 and 660ppm. Figure 2 and 3 depict the corrected mortality rates observed after 24, 48, and 72 hours of exposure to the tested extracts. These results clearly demonstrate a concentration-dependent increase in mortality, with a corresponding rise in exposure duration. The lethal concentrations LC_{25}, LC_{50}, and LC_{90}, along with other relevant parameters, were calculated and are presented in Table 3. Notably, both methanolic extracts of *L. dentata* and *N. oleander* exhibited higher toxicity against *C. longiareolata*

Table 1. Phytochemical composition of *L. dentata* and *N. oleander*

Phytochemical compounds	*Lavandula dentata*	*Nerium oleander*
Flavonoids	++	++
Tannins Gallic	++	++
Tannins Catechic	++	++
Terpenes	++	++
Alkaloids	–	–
Sterols	++	–
Saponins	–	++
Quinones	++	–

(++): Abundance; (+): presence; (–): absence.

Table 2. Yield of the two extracts of *L. dentata and N. oleander*

Plant	*L. dentata*		*N. oleander*	
Extract	Aqueous	methanolic (70%)	Aqueous	methanolic (70%)
Yield %	6.06%	16.16%	6%	15%

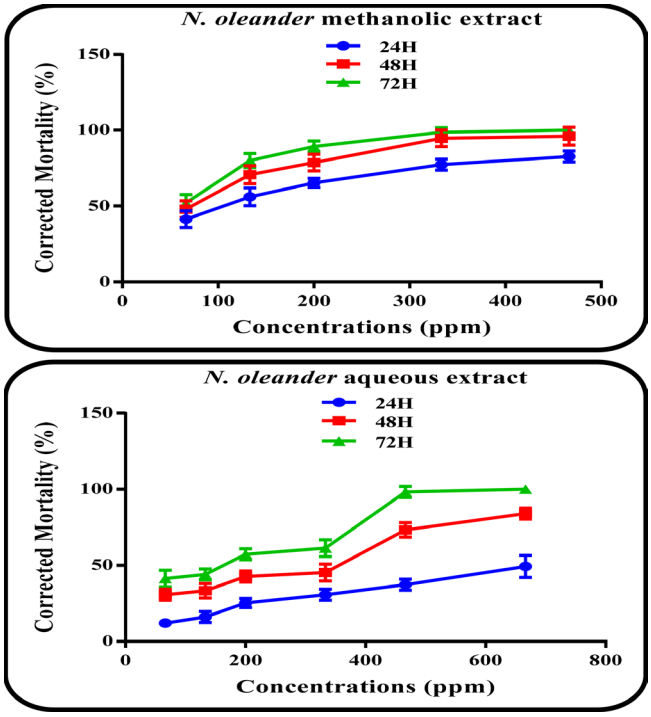

Figure 2. Larvicidal activity of *N. oleander* aqueous and methanolic extracts against *C. longiareolata* at different exposure times.

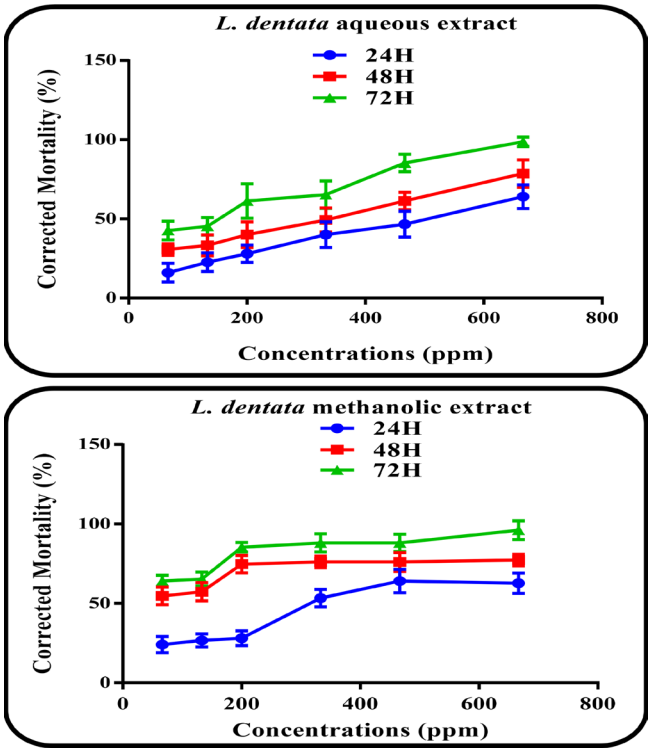

Figure 3. Larvicidal activity of aqueous and methanolic extracts of *L. dentata* against *C. longiareolata* at different exposure times.

Table 3. Toxicity of *L. dentata* and *N. oleander* applied on the larvae of *C. longiareolata*: Determination of Lethal and sub lethal concentrations (ppm)

Plant extract	Exposure time (hours)	LC_{25}	LC_{50}	LC_{90}	Hill slope	R^2
L. dentata aqueous extract	24	156.7	463.8	4065	1.118	0.963
	48	75.74	261.6	3120	0.886	0.881
	72	44.28	121.8	922	1.086	0.841
L. dentata methanolic extract	24	44.28	338.1	3258	0.969	0.884
	48	6.103	48.24	3010	0.531	0.828
	72	12.37	41.46	465.8	0.908	0.851
N. oleander aqueous extract	24	221.3	760.9	9000	0.889	0.977
	48	81.86	234.85	1925	1.044	0.828
	72	53.92	130.4	763.2	1.244	0.765
N. oleander methanolic extract	24	32.42	99.64	991.3	0.978	0.995
	48	34.92	72.4	311.7	1.506	0.979
	72	37.24	64.64	194.73	1.992	0.992

compared to their aqueous counterparts. Specifically, the LC_{50} values for *N. oleander* methanolic extract were 99.64, 72.4, and 64.64ppm for the respective time intervals of 24, 48, and 72 hours, whereas for *L. dentata* methanolic extract, the LC_{50} values were 338.1, 48.24, and 41.46 ppm, respectively.

Effect of methanolic and aqueous extract of *L. dentata* and *N. oleander* on enzyme biomarkers

The impact of methanolic and aqueous extracts from *L. dentata* and *N. oleander* on enzyme biomarkers was examined. These extracts were administered to newly molted L_4 larvae of *C. longiareolata* at two lethal concentrations (25 and 50), alongside a control group. The effects of these extracts were assessed at various time points (24, 48, and 72 hours post-treatment). Both detoxification biomarker, glutathione S-transferases (GSTs), and oxidative stress biomarker, catalase, were evaluated. Results were normalized to protein levels (mg) determined from a reference curve. Multiple comparisons of means via Dunnett's test revealed a significant rise in GSTs' specific activity in series treated with methanolic and aqueous extracts from both plants and at both concentrations (LC_{25} and LC_{50}) across different treatment durations (24, 48, and 72 hours) compared to the control group (See Figure 4 and 5). However, the specific activity of GSTs decreased after 48 and 72 hours of treatment with the methanolic extract of *N. oleander*.

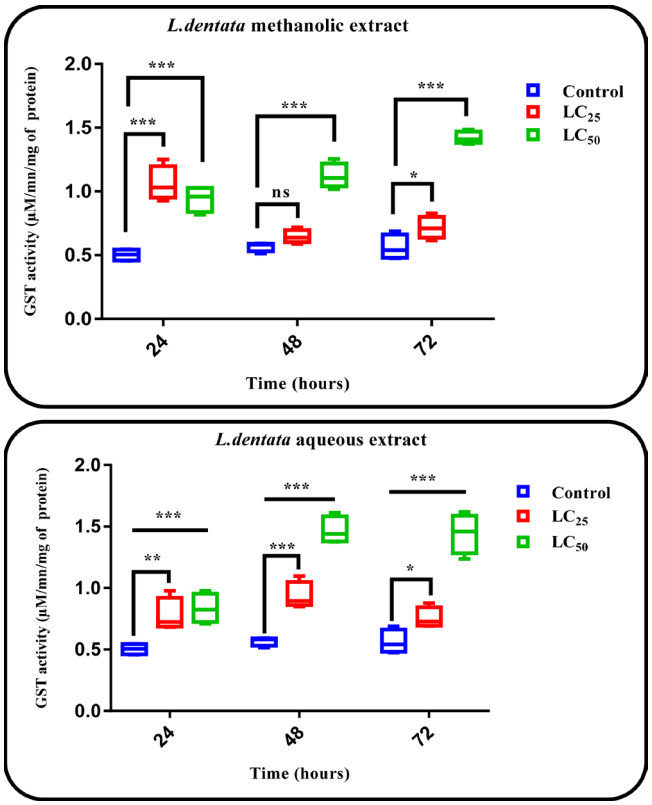

Figure 4. Effect of methanolic and aqueous extract of *L. dentata* (LC_{25} and LC_{50}) on the specific activity of GSTs (µM/mn/mg of protein) in *C. longiareolata* (m±SD, n=4). (ns non-significant (p≥0.05);* Difference significant (p<0.05); ** Difference highly significant (p<0.01); *** Difference very highly significant (p<0.001) between control and treated series).

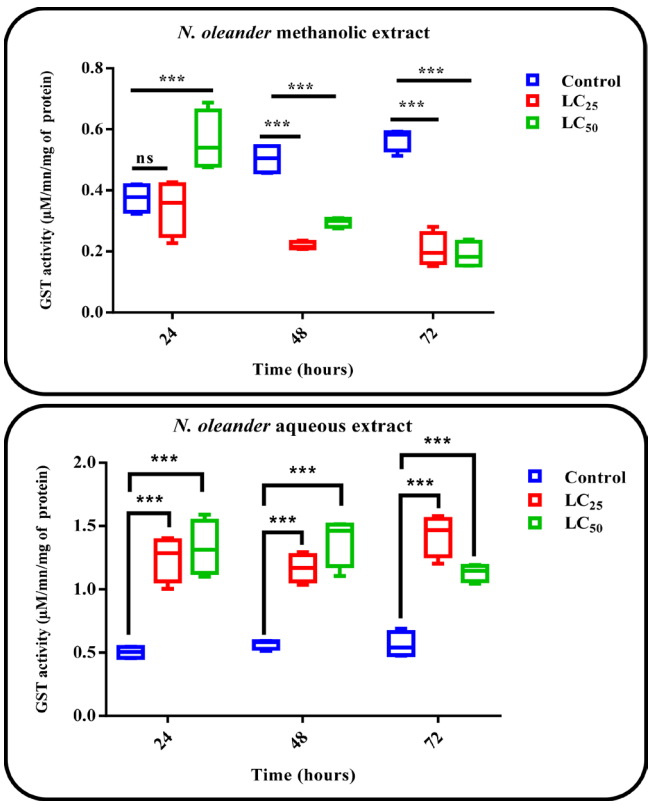

Figure 5. Effect of methanolic and aqueous extract of *N. oleander* (LC_{25} and LC_{50}) on the specific activity of GSTs (µM/mn/mg of protein) in *C. longiareolata* (m±SD, n=4). (ns non-significant (p≥0.05); *** Difference very highly significant (p<0.001) between control and treated series).

Dunnett's multiple comparison test was conducted to compare the means of catalase specific activity between the control and treated groups at LC_{25} and LC_{50} concentrations using methanolic extract of *L. dentata*. The results indicated a significant increase after 24 hours (p=0.257, p=0.002), followed by a non-significant change after 48 hours (p=0.209, p=0.200), and a highly significant decrease after 72 hours compared to the control group. Moreover, catalase activity showed a significant increase after 24 and 48 hours in the treated groups at LC_{25} and LC_{50} concentrations with the aqueous extract of *L. dentata* compared to the control, followed by a reduction after 72 hours of treatment (refer to Figure 4). Similarly, the results showed an increase in catalase activity after 24 hours in the treated groups with methanolic and aqueous extracts of *N. oleander* at LC_{25} and LC_{50} concentrations, followed by a highly significant reduction after 72 hours compared to the control group (refer to Figure 6 and 7).

Effect of extracts on growth of *Culiseta longiareolata*

The impact of aqueous and methanolic extracts from *L. dentata* and *N. oleander* (at LC_{25} and LC_{50} concentrations) on the growth of newly molted fourth instar larvae of *Culiseta longiareolata* was investigated across various treatment durations (24, 48, and 72 hours). Comparison of mean values between the control and treated groups revealed that the LC_{25} concentrations of methanolic and aqueous extracts from both *L. dentata* and *N. oleander* had no significant effect on the body weight of *C. longiareolata* larvae at different treatment intervals (p>0.05), except for the methanolic extract of *N. oleander*, which significantly reduced weight after 48 and 72 hours (p=0.014; p=0.028). However, this parameter was notably influenced by the LC_{50} concentrations at 24 hours (p=0.049; p=0.011; p=0.010; p=0.058), 48 hours (p=0.003; p=0.004; p=0.000; p=0.005), and 72 hours (p=0.004; p=0.005; p=0.000; p=0.006) with both methanolic and aqueous extracts from *L. dentata* and *N. oleander*, respectively (see Figure 8 and 9).

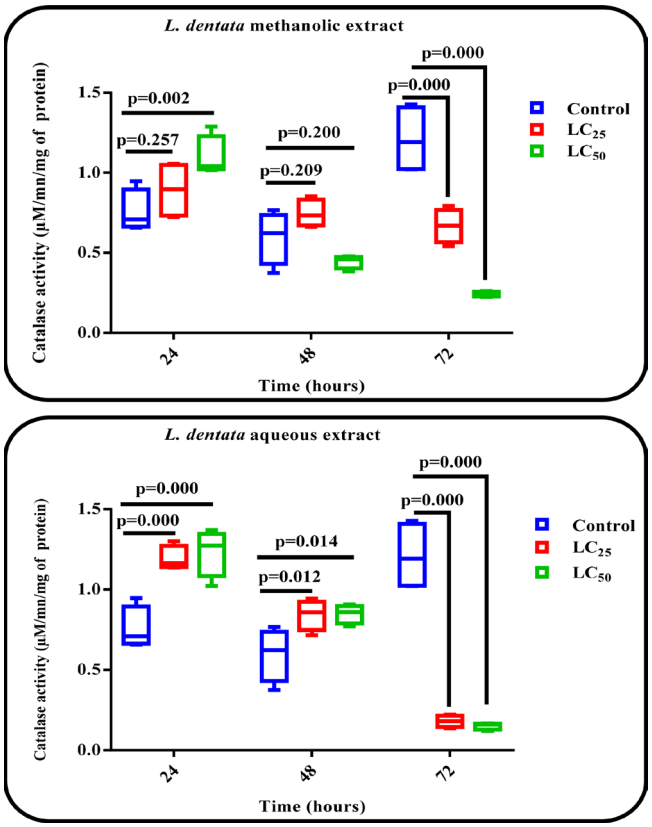

Figure 6. Effect of methanolic and aqueous extract of *L. dentata* (LC_{25} and LC_{50}) on specific catalase activity (µM/mn/mg of protein) in *C. longiareolata* (m ± SD, n=4).

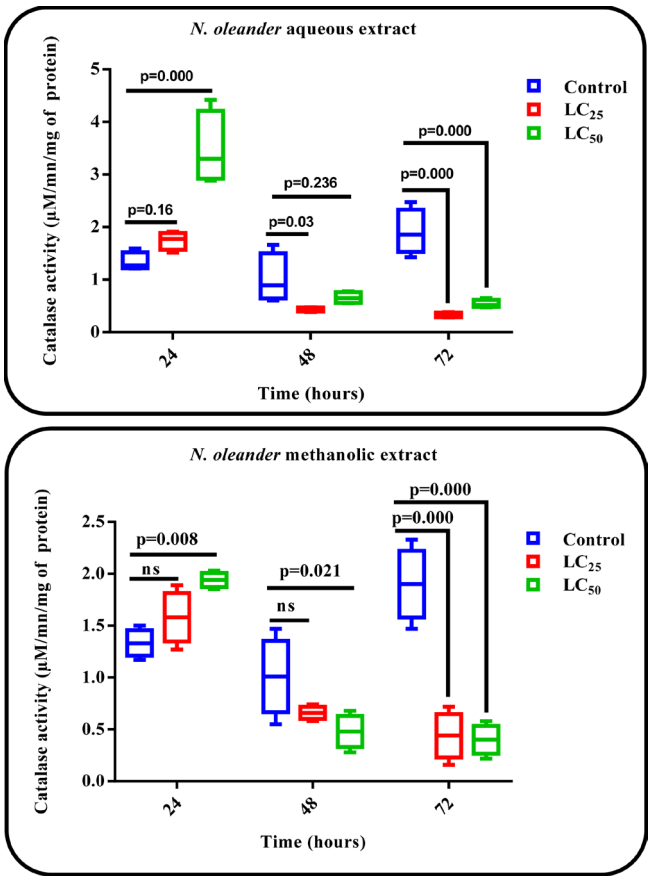

Figure 7. Effect of methanolic and aqueous extract of *N. oleander* (LC_{25} and LC_{50}) on specific catalase activity (µM/mn/mg of protein) in *C. longiareolata* (m ± SD, n=4).

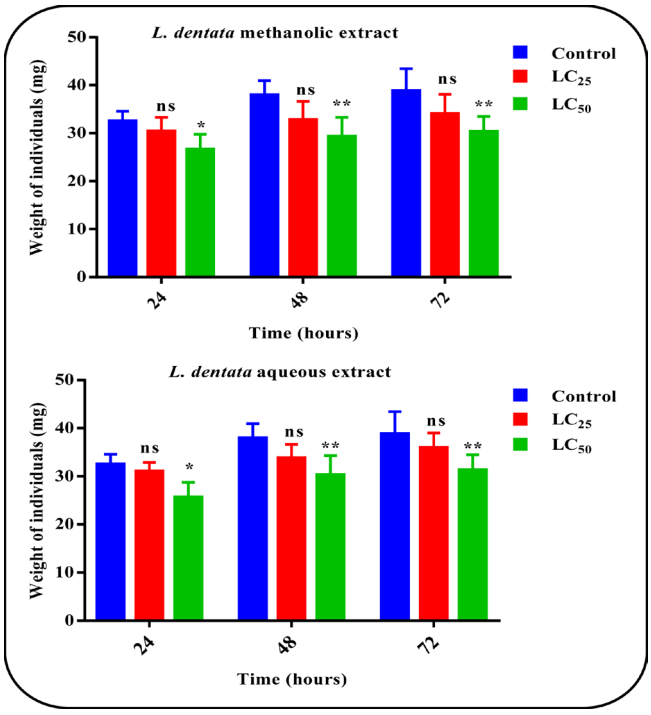

Figure 8. Effect of methanolic and aqueous extract of *L. dentata* (LC_{25} and LC_{50}) on the weight (mg) of 4th instar larvae in *Culiseta longiareolata* (m ± SD, n = 4) (ns non-significant (p≥0.05); * Difference significant (p<0.05); ** Difference highly significant (p<0.01) between control and treated series).

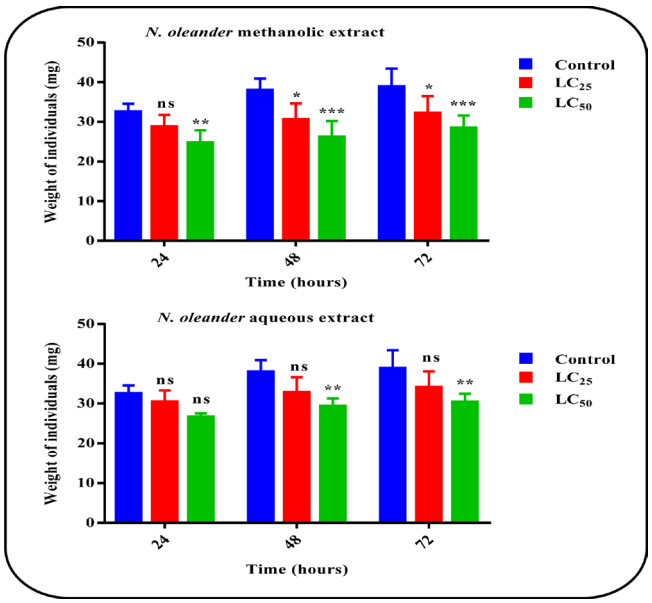

Figure 9. Effect of methanolic and aqueous extract of *N. oleander* (LC_{25} and LC_{50}) on the weight (mg) of 4th instar larvae in *Culiseta longiareolata* (m ± SD, n = 4)(ns non-significant (p≥0.05); * Difference significant (p<0.05); ** Difference highly significant (p<0.01); *** Difference very highly significant (p<0.001) between control and treated series).

Impact of extracts on the biochemical composition of mosquitoes

The influence of methanolic and aqueous extracts derived from *L. dentata* and *N. oleander* (at LC_{25} and LC_{50} concentrations) on the biochemical composition of fourth instar larvae of *C. longiareolata* was examined, assessing proteins, carbohydrates, and lipids at various time points (24, 48, and 72 hours post-treatment). The results detailing the impact of different treatments on protein content are provided in Table 4. Analysis of variance indicated significant differences among treatment groups. Dunnett's test conducted between the control and treated groups revealed a highly significant decrease in protein levels (p=0.000) following treatment with all extracts at both concentrations. Furthermore, as depicted in Table 4, there was a substantial decrease in carbohydrate content post-treatment with various extracts across all time points (p=0.000), except for the LC_{25} treated series with methanolic extract of *L. dentata*, which demonstrated a significant reduction (p=0.0108) in total carbohydrates after 72 hours compared to the control series. Regarding lipid levels, all treated larvae exhibited a significant decrease in lipid content (p=0.000) compared to the control group, except for the LC_{25} treated series with *N. oleander* aqueous extract, which showed a significant reduction (p=0.011) after 72 hours of treatment.

DISCUSSION

Extraction stands as a crucial step in isolating, identifying, and utilizing phenolic compounds, and it lacks a singular standard method (Ignat *et al*., 2011). The reported yield of extracts in our study was influenced by the chosen solvent for extraction, with methanol extracts yielding higher quantities compared to aqueous ones. Methanol generally proves more effective in extracting lower molecular weight polyphenols, while the combined use of water and organic solvents can enhance the extraction of chemicals soluble in both mediums (Do *et al*., 2014). The biological effect was attributed to major compounds found in *N. oleander* including gallic acid, 4-Hydroxy benzoic acid, vanillic acid, p-coumaric acid, jasmonic acid, rutin, o-cresol, and 3,4-xylenol (Khouchlaa *et al*., 2020). Indeed, *L. dentata* contains many classes of bioactive compounds, including flavonoids, terpenoids, tannins, and anthracene derivatives (Bouyahya *et al*., 2023)

In the larvicidal study, both methanolic flower extracts of *L. dentata* and *N. oleander* exhibited greater toxicity against fourth instar larvae of *C. longiareolata* compared to their aqueous counterparts, with LC_{50} values of 338.1 and 99.64 ppm, respectively, after 24 hours of treatment. Mortality increased with prolonged exposure time, possibly due to the presence of polyphenols in the methanolic extracts. Notably, there is limited existing literature on the larvicidal activity of methanolic and aqueous extracts of *L. dentata* and *N. oleander* against *C. longiareolata.* El-Akhal *et al*. (2015) reported that ethanolic extracts of *Nerium oleander* applied against *Culex pipiens* larvae resulted in lethal concentrations LC_{50}

Table 4. Effect of methanolic and aqueous extract of *L. dentata* and *N. oleander* (LC_{25} and LC_{50}) on protein, carbohydrate and lipid content (µg/individual) in *C. longiareolata* larvae (m ± SD, n=4)

Biochemical component	Plant	Time (hours)	Control	Methanolic extract		Aqueous extract	
				LC_{25}	LC_{50}	LC_{25}	LC_{50}
Proteins	*L. dentata*	24	154.3±16.06^a	74.96±3.49^b	67.54±4.71^b	94.55±12.1^b	55.79±5.6^b
		48	215.71±16.13^a	99.74±4.40^b	54.96±4.77^b	77.46±10.8^b	52.63±5.3^b
		72	181.46±2.84^a	78.04±12.5^b	30.29±1.90^b	97.21±13.1^b	34.3±2.37^b
	N. oleander	24	154.3±16.06^a	122.88±6.57^b	86.05±5.12^b	76.21±8.81^b	62.29±6.7^b
		48	215.71±16.13^a	160.21±3.72^b	77.29±13.1^b	105.18±21^b	57.95±5.2^b
		72	181.46±2.84^a	116.05±3.71^b	82.71±4.72^b	105.74±18^b	83.62±7.2^b
Carbohydrates	*L. dentata*	24	115.08±12.04^a	61.08±18.74^b	49.54±6.91^b	61±5.60^b	24.66±4.8^b
		48	109.87±8.26^a	80.87±10.88^b	67.95±1.92^b	73.16±9.05^b	33.44±1.6^b
		72	110.33±4.36^a	89.41±7.31^b	58.54±8.12^b	39.94±11.8^b	23.66±4.2^b
	N. oleander	24	115.08±12.04^a	24.12±4.44^b	35.25±12.7^b	69.77±9.67^b	61.38±9.3^b
		48	109.87±8.26^a	27.37±3.9^b	35±11.21^b	67.05±5.58^b	57.6±10.8^b
		72	110.33±4.36^a	51.95±5.66^b	36.54±2.65^b	71.16±5.84^b	67.5±11.9^b
Lipids	*L. dentata*	24	205.54±4.38^a	137.17±9.54^b	136.87±3.4^b	168.88±9.5^b	109.3±15.8^b
		48	257.66±2.9^a	173.01±10.6^b	112.74±10^b	100.3±18.7^b	113.4±12.7^b
		72	214.65±3.90^a	94.61±5.65^b	94.21±2.24^b	92.31±9.85^b	97.85±9.68^b
	N. oleander	24	205.54±4.38^a	127.58±4.07^b	74.84±11.6^b	207.35±5.8^b	156.51±4.9^b
		48	257.66±2.9^a	152.77±1.07^b	135.35±4.5^b	256.43±5.7^b	117.7±23.7^b
		72	214.65±3.90^a	185.16±17.5^b	162.16±26^b	193.9±13.3^b	110.9±3.37^b

and LC_{90} of 57.57 mg/mL and 166.35 mg/mL, respectively. Raveen *et al*. (2014) found that hexane flower extract of *N. oleander* exhibited high larvicidal activity against *Culex quinquefasciatus* with LC_{50} values of 102.54 and 61.11 ppm after 24 and 48 hours, respectively. Additionally, aqueous leaf extract displayed larval toxicity against various instar larvae and pupae of *Anopheles stephensi* with LC_{50} values ranging from 232.90 to 369.96 ppm (Roni *et al*., 2013).The results of Bouabida and Dris (2022a) indicated that the methanolic extracts of three plants *Ruta graveolens, Ruta montana* and *Artemisia absinthium* possess insecticidal activity against mosquito vectors of avian plasmodium (*Culiseta longiareolata*) with LC_{50} of 43.24, 97.74 and 199.5 ppm respectively. In addition, The *Ruta graveolens* hydro-methanolic extract has a very effective larvicidal activity with LC_{50} of 33.06, 37.08, 82.27 and 150.6 mg/L for first, second, third and fourth instar larvae of *Culiseta longiareolata* respectively (Dris *et al*., 2021). The toxicity of aqueous extracts of six plants *Ambrosia maritima, Hertia centifolia*, and *Xanthium strumarium* (Asteraceae), *Daturas tramonium* and *Solanum elaeagnifolium* (Solanaceae) and *Salvea verbena* (Lamiaceae) was tested on *C. Longiareolata* by Belkhiri *et al.* (2021). The results showed a strong positive correlation between the mortality rates recorded and the concentration of the extract used against mosquitoes.

Glutathione S-transferase (GST) activity plays a vital role in detoxifying oxidative stress products and conjugating glutathione to xenobiotic metabolites for excretion, while catalase is considered among the antioxidant enzymes crucial for insect antioxidant defense (Kiran & Prakash, 2015; Osioma & Ejoh, 2021). The observed significant increase in GST and catalase activities in fourth instar larvae of *C. longiareolata* treated with different extracts compared to controls suggests an establishment of detoxification processes as a defense mechanism against the pesticide. Several studies have reported increased levels of detoxifying enzymes in mosquito larvae exposed to pesticides or plant extracts (Tripathy *et al*., 2011; Karthi *et al*., 2020; Shahat *et al*., 2020; Amala *et al*., 2021).

An insecticide is detoxified by one or more enzymes before reaching its site of action. Resistance may develop to a single synthetic insecticide such as organophosphates, carbamates, pyrethroids, and neonicotinoids. But, plant extract-based pesticides contain numerous ingredients, making resistance development more challenging (Poonsri *et al*., 2019).Tested plant extracts (LC_{50}) resulted in reduced weight of fourth instar larvae in *Culiseta longiareolata*. Similarly, Bouabida and Dris (2022a) reported that methanolic extracts from *Ruta graveolens, Ruta montana* and *Artemisia absinthium* affected growth in post-embryonic stages of *C. longiareolata*. Additionally, sublethal concentrations (LC_{25} and LC_{50}) of each tested extract significantly decreased total protein, carbohydrate, and lipid contents of *C. longiareolata* fourth larval instar compared to the control group. Several studies have attributed this decline in biochemical composition contents to treatment of mosquito larvae with plant extracts (Bouabida & Dris, 2020; Shahat *et al*., 2020; Dris & Bouabida, 2023). Depletion in carbohydrate content may result from utilization of larval tissue's reserved carbohydrate sources under plant extract-induced stress (Shehata, 2018). Similarly, reduction in lipid content indicates a negative effect of extracts on lipid metabolism and peroxidation (Sharma *et al*., 2009).

CONCLUSION

In light of the results obtained, methanol extracts exhibited superior yield compared to aqueous extracts. Methanol extracts of both plants demonstrated higher toxicity against fourth instar larvae of *C. longiareolata* compared to their aqueous counterparts. Furthermore, the application of methanolic and aqueous flower extracts of *L. dentata* and *N. oleander* led to alterations in detoxification enzymes and larval growth, consequently causing changes in the biochemical composition of the larvae. Our findings hold potential for contributing to the establishment of a database for natural plant-based larvicidal studies, which could be instrumental in future large-scale initiatives aimed at vector mosquito control.

ACKNOWLEDGEMENTS

This work was supported by Echahid Cheikh Larbi Tebessi University, Tebessa, Algeria and the Ministry of High Education and Scientific Research of Algeria (PRFU Project D01N01UN12012230004) to Dr. D. DRIS.

Conflict of Interest

The author declares that they have no conflict of interest.

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